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Comparative assessment of the sensitivity of oilseed rape and wheat to limited water supply

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Title: Comparative assessment of the sensitivity of oilseed rape and wheat to limited water supply

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Key words: oilseed rape, root hydraulic conductivity, water stress, wheat

Abbreviations:

DAS – days after sowing, DW – dry weight, FC - field capacity, FW – fresh weight, OSR – oilseed rape, PWP – permanent wilting point, RLD – root length density, WUE – water use efficiency, g_s – stomatal conductance.

Abstract

The drought-sensitivity of oilseed rape (OSR, *Brassica napus* cv. SW Landmark) was investigated, using the more widely studied crop species wheat (*Triticum aestivum* cv. Tybalt) as a benchmark. The water relations of OSR and wheat were compared in lysimeter and controlled environment experiments to test the hypothesis that the growth of OSR is restricted to a greater extent by soil drying than wheat and to determine whether the greater sensitivity results from differences in root or shoot traits. Plants were grown, with or without irrigation, in 1.2 m tall lysimeters packed with a sandy clay loam soil. The experiment was conducted in an open-sided glasshouse to encourage air flow and to resemble a field environment as far as possible; plant population densities were equivalent to commercial crops. Irrigated OSR (evapo)transpired more water than wheat (498 vs 355 mm), but had a comparable water use efficiency (WUE; 4.1 vs 4.4 g DW mm⁻¹ H₂O). Oilseed rape showed a greater reduction in above-ground growth (52% vs 21%) and a smaller increase in WUE (27% vs 45%) when water was withheld. Oilseed rape also responded to soil drying at a lower soil moisture deficit than wheat; transpiration rates fell below the potential of irrigated plants when plant available water remaining in the soil profile declined from 54 to 23% compared to 38-9% for wheat. The root hydraulic conductivity of young OSR plants, measured on root surface area basis, was about twice that of wheat, and was comparable on a root length basis. The results show that OSR was more sensitive to a restricted water supply than the benchmark species wheat and that the greater sensitivity resulted from differences in shoot, rather than root, characteristics. The root system of OSR was at least as efficient as wheat at extracting water from soil.

Introduction

Oilseed rape (OSR; mostly *Brassica napus*, *B. rapa* and *B. juncea*) is a crop of major global significance. In 2011, 62 Mt of rapeseed were produced from a cropped area of 34 million ha (FAOSTAT 2013). Europe was the major centre of production followed by Canada, China, India and Australia, which between them accounted for 96% of the world's total. There is concern in some countries that in spite of improvements in the yield potential of new varieties, farm yields of OSR have not improved for a number of years (Berry & Spink 2006). It has been speculated that poor soil management, inadequate root growth and a restricted water supply may be contributory factors (Berry & Spink 2006). With climate change models predicting hotter and drier summers for much of the current production area, greater restrictions on crop water supply are likely in the future (Christensen *et al.* 2011; Stocker *et*

al. 2013). It is important, therefore, to establish agronomic practices and to develop varieties that are better able to avoid or tolerate periods of drought.

Boyer (2010) has argued that progress towards improving the drought avoidance and tolerance of crops could be accelerated if benchmark measurements were included in experimental designs allowing experiments to be repeated and results to be compared across studies. We suggest that benchmarking between species is also valuable. Currently our understanding of the water relations of OSR is poor by comparison with other species. Comparing the response of OSR to restricted water supply with that of a more widely studied species such as wheat would enable the relative sensitivity of OSR to drought to be evaluated. The use of wheat as the benchmark is particularly relevant, because in many production systems wheat and OSR are grown in rotation, and are therefore subject to the same edaphic and climatic conditions. Information on the relative sensitivity of different crop species to drought when grown under the same conditions, and an understanding of the physiological mechanisms underlying any differences in response, would assist growers in making decisions about the choice of crop species and management options for sites of known drought risk. Benchmarking the physiological responses of OSR to soil drying against those of wheat would also enable crop simulation models developed for wheat to be adapted and parameterised for OSR so that the likely yield and economic benefits of improvements in particular traits can be estimated in advance of any concerted breeding effort.

Uptake of water by plants and crops is driven by the evaporative demand of the atmosphere; where uptake exceeds precipitation and recharge of the rooting profile from ground water, a soil water deficit develops. The response of plants to soil drying includes a reduction in leaf expansion, the closure of stomata, and the acceleration of leaf senescence and abscission, which helps conserve the remaining water and prevents tissue dehydration, although it also reduces CO₂ assimilation and hence productivity (Jensen *et al.* 1998; Jones 1998; Pic *et al.* 2002). Hydraulic and chemical signals have been implicated in the control of these responses to drought, but the precise mechanisms are still unclear (Dodd 2005; Parent *et al.* 2009; Sperry *et al.* 2000; Tardieu & Davies 1993; Voisin *et al.* 2006).

Theoretical considerations and empirical studies have given rise to the concept that there is a critical minimum root length density (RLD, root length per unit volume of soil) for easy extraction of plant available water (Gregory & Brown 1989; King *et al.* 2003; Passioura 1983; Van Noordwijk 1983). Thus, for cereal crops an RLD of 1-2 cm cm⁻³ is considered sufficient for extraction of most of the available water (*i.e.*, that held between -0.005 and -1.5 MPa). Although root length densities of cereals typically exceed this value in the top-soil, those in the sub-soil are often less, suggesting that increasing RLD in the subsoil could increase the ability of crops to access soil water reserves (King *et al.* 2003; Lilley & Kirkegaard 2011). However, species differ markedly in their root anatomy and some are able to extract water efficiently (when it is available) with a smaller RLD as a result of their greater hydraulic conductance (Gallardo *et al.* 1996). The RLD of OSR and its

distribution down the soil profile is broadly comparable with that of wheat, although there is evidence of considerable variation in root growth of OSR across farms and cultivation systems (Blake & Spink 2005; Blake *et al.* 2006). However, the implications of these root growth patterns for water uptake cannot be determined, because little is known about the hydraulic properties of OSR roots.

Species also differ in the response of their shoots to soil water deficits (Tardieu *et al.* 1996). A conservative response in which stomatal closure is elicited early during soil drying to maintain leaf water potential may be an undesirable characteristic for production environments where soil water supply is reasonably predictable, as this could restrict carbon assimilation even though there may be enough soil water for the crop to complete seed production. In less conservative species, osmotic adjustment can help maintain a positive turgor pressure in spite of a decline in leaf water potential (Taiz & Zeiger 1998). There is some evidence that OSR has only a limited capacity to adjust osmotically during the onset of water stress and thus may be relatively conservative in its response to soil drying (Jensen *et al.* 1996; Muller *et al.* 2010), but to our knowledge a direct comparison with other species, such as wheat has not been made.

The objective of the two experiments reported here was to determine the relative sensitivity of the OSR species *B. napus* to restricted water supply by comparing it to the benchmark species wheat. In the first experiment, the effects of a restricted water supply on growth and transpiration of the two species were compared. In a second experiment, root hydraulic conductivity of OSR and wheat were compared.

Materials and methods

Experiment I - Response to restricted water supply

Experimental design and treatments

An experiment involving 20 lysimeters laid out in a randomised block design was set-up in April 2007 in a glasshouse at SRUC's Easter Bush facility, Penicuik, Scotland. The glasshouse was unheated and open-sided to encourage airflow to resemble field conditions. Treatments were species (OSR and wheat) and irrigation regime (irrigated and non-irrigated) with five replications per treatment. In addition, four unplanted lysimeters (two irrigated and two non-irrigated) were set up for measurement of soil hydraulic properties and allocated at random to two of the main experimental blocks.

The lysimeters were constructed of polyvinyl chloride (PVC) pipes of 30 cm internal diameter and 120 cm height. A sandy clay loam soil (MacMerry series, Vinten *et al.* 1994) was packed into the lysimeters to within 15 cm of the upper rim giving a gradient of increasing bulk density down the soil profile. The top 28 cm was at a mean (\pm sem) dry bulk density of $1.11 \pm 0.025 \text{ g cm}^{-3}$, the middle 40 cm section was $1.15 \pm 0.031 \text{ g cm}^{-3}$ and the bottom 37 cm was $1.20 \pm 0.034 \text{ g cm}^{-3}$ (measured at the end of the experiment). A five cm diameter access tube for a capacitance probe (Sentek diviner 2000, Kent Town, Australia) was placed into the centre of the lysimeter before packing the soil and the soil was packed around it. After packing, the soil was irrigated thoroughly to exceed field capacity (FC) and its surface covered with plastic sheeting to minimise evaporation; the soil was then allowed to drain for three days before sowing on 30 April.

Spring oilseed rape (*Brassica napus* L. cv. SW Landmark) was sown and thinned after plant emergence to give a population of eight plants per lysimeter ($113 \text{ plants m}^{-2}$) and spring wheat (*Triticum aestivum* L. cv. Tybalt), at a population of 38 plants per lysimeter ($552 \text{ plants m}^{-2}$). These varieties were chosen because they have similar life-cycle duration. Nitrogen fertilizer (NH_4NO_3) was applied to the soil surface at a rate of 100 kg ha^{-1} nitrogen (typical for field crops) four days after sowing (DAS). P (60 kg ha^{-1}), K (35 kg ha^{-1}) and S (14 kg ha^{-1}) had been incorporated into the top 28 cm of soil prior to packing and sowing.

The soil moisture measurement made on the day of sowing was taken to be the moisture content at FC and used as a reference for determining soil moisture deficit. All lysimeters were irrigated over the first 14 DAS to enable seedlings to establish and to wash N fertilizer into the soil. For plants in the non-irrigated treatment, water was withheld from 14 DAS. Plants in the irrigated treatment were given water once per week to return the soil to 90% of FC. This irrigation strategy ensured that depletion of plant available water between irrigation events never exceeded 60% for wheat, and only exceed 60% in OSR in the final

two weeks of the experiment.

At 49 DAS, mesh sleeves (Netlon 3 mm mesh, Conwed Plastics, Genk, Belgium) were placed around the canopies to simulate the presence of neighbouring plants in a field situation, thereby reducing possible edge effects. Oilseed rape had on average four unfolded leaves per plant and wheat on average five per plant at this point in time. The sleeve was raised at regular intervals so that it was maintained at canopy height. Plants were treated with fungicides and insecticides when appropriate to control powdery mildew, aphids and flea beetle. The experiment lasted 84 days for wheat and 85 days for OSR.

Measurements

Air temperature and relative humidity were logged every hour at plant height with a DL3000-8.10 logger (Delta-T Devices Ltd, Cambridge, UK) positioned at the edge of block two of the experiment. Volumetric soil moisture content of the upper 80 cm of soil was measured in 10 cm depth intervals every Thursday and Friday during the experiment with a capacitance probe (Sentek Diviner 2000, Sentek Pty Ltd, Kent Town, Australia). After the Thursday measurement, the lysimeters in the irrigated treatment were watered, before measuring again the following day.

The rate of evaporation from the soil surface under the canopy was determined using micro-lysimeters (Boast & Robertson 1982; Daamen *et al.* 1993). Open ended tubes (100mm x 22mm id) were inserted into the soil, a core of soil was removed, weighed within its tube, and then returned to the hole created by the coring in the main lysimeter after first sealing the bottom end of the tube with plastic film. The micro-lysimeters were re-weighed without the plastic film after 6 days. The difference in weight equalled the water loss via evaporation from the soil.

The height of three tagged plants per lysimeter was measured once a week to the nearest half centimetre, from the soil surface to the tip of the tallest (outstretched) leaf. On 74 DAS, the stomatal conductance (g_s) of the youngest fully expanded leaf in the top of the canopy of one plant per lysimeter was measured around midday with a portable IRGA (ADC-LCA4 Analytical Development Co. Ltd, Hoddesdon, Herts, U.K.).

On 84 DAS for wheat and 85 DAS for OSR the plants were destructively harvested. This was during the grain and seed filling period respectively rather than at grain and seed maturity because measurements of leaf relative water content (RWC) and root length were required before tissue had senesced. On the day of harvest, the youngest fully expanded leaf was excised from one plant in each lysimeter for determination of RWC (adapted from Gallardo *et al.* 1996). A rectangular segment was cut from the leaf, avoiding large veins. The segment was weighed (FW), floated on deionised water in the dark at 21°C for 3-4 hours to attain full turgor, reweighed, oven dried and the dry weight (DW) determined. RWC was

calculated as $(FW - DW) / (Turgid\ Weight - DW)$.

After counting total shoot numbers and determining the total fresh weight of leaves, ears and pods and stems combined, a subsample (a random 50% for OSR and 25% for wheat) was taken for further analysis. The leaf, pod or ear and stem area of the subsample were determined with a LI-3100 leaf area meter (Li-Cor Biosciences, Cambridge, UK). The dry weights of the plant parts were determined after drying to constant mass in a fan-assisted oven at 80°C. The total plant DW per lysimeter was calculated after accounting for the sub-sampling, by using the ratio of the fresh weight of the total sample to its subsample.

Root samples were taken after the lysimeters were laid down horizontally and cut open with a saw. Soil cores with a volume of 209 cm³ were taken at between 30-40 and 70-80 cm soil depths avoiding the outer edge of the lysimeter. Soil was washed from the roots with a Delta-T root washer (Delta-T Devices Ltd, Cambridge, U.K.) and the roots collected on a 0.5 mm mesh. After manually removing organic debris, root samples were immersed in a film of water within a clear plastic tray, spread out to minimize overlap and subsequently scanned with a Régent LA1600 scanner. Images were analysed with Winrhizo software (Régent Instruments Inc, Quebec, Canada).

Soil cores from three unplanted lysimeters were taken to determine soil moisture release curves at the contrasting bulk densities (Rowell 1994). The water content at -1.5 MPa matric potential was taken to be the permanent wilting point (PWP) (Rowell 1994). Although some plant species can absorb water from soil at potentials much lower than this; the amount of water actually held by the soil below a matric potential of -1.5 MPa is small (Kirkham 2004).

Calculations

Vapour pressure deficit (VPD) is the difference between the saturation vapour pressure (e_s) and the actual vapour pressure (e_a). Vapour pressure deficit is often regarded as a measure of the 'drying power' of the air, because it plays an important part in determining the relative rates of growth and transpiration in plants (Monteith & Unsworth, 2013). VPD was calculated for every hour and the 24 hour average (from midnight to midnight) was calculated and plotted in Fig 1. Vapour pressure deficit (VPD) was calculated using the following equations:

$$VPD = e_s - e_a$$

$$e_s(T) = e_s(T^*) \exp[A(T-T^*)/(T-T')] \quad (\text{equation 2.27 in Monteith \& Unsworth,}$$

2013),

where $e_s(T)$ = saturation vapour pressure in kPa, $A = 17.27$, $T^* = 273K$, $(e_s(T^*) = 0.611\text{ kPa}$, and $T' = 36K$, T = air temperature in Kelvin at canopy height.

$$e_a = (RH e_s)/100$$

where RH is relative humidity of the air at canopy height.

Total plant available water to 80 cm soil depth was calculated as that held between FC and PWP. Evaporation of water from the soil surface was calculated using micro-lysimeter data and deducted from the total water loss from 0 to 80 cm of soil to give the transpiration rate. This widely adopted method for indirectly estimating transpiration rate assumes that the capacitance of the crop is negligible relative to the amount of water transpired. Evaporation data from micro-lysimeters were available for the periods DAS 32-38, 39-45, 46-52 and 60-66, and evaporation under the canopy in other intervals was interpolated from these data. The trend in evaporation rates measured with micro-lysimeters was comparable to that derived from measurements of volumetric soil moisture content of unplanted irrigated and non-irrigated lysimeters made using the capacitance probe.

Volumetric soil moisture data, obtained with the capacitance probe, were used to estimate cumulative water uptake from each 10 cm soil layer over time. Curves of soil moisture content over time for individual depth intervals were sigmoid in shape with the soil moisture content remaining at FC for a period before declining at a near linear rate to a minimum value. To interpolate between measurement dates and obtain an estimate for the onset of water uptake from a particular depth, a straight line was fitted through the first three points at which the line started to deviate from FC, and the date at which this line intersected the horizontal (soil moisture content at FC) was taken to be the onset of water extraction. Inflow rates per unit root length and root surface area for irrigated lysimeters were calculated from rates of water extraction from 30-40 cm and 70-80 soil depths between DAS 73 and DAS 80 and the root length and surface area measured at final harvest (DAS 84 - 85).

Water use efficiency (WUE) was calculated by dividing the above ground biomass at the end of the experiment (DAS 84-85) by the total water use in mm (transpiration and soil evaporation combined) per unit ground area in m². For non-irrigated lysimeters, adjusted values of WUE were also calculated as the above ground biomass at the end of the experiment divided by cumulative evapotranspiration (mm) until plant available water was exhausted. This accounts for the possible overestimation of soil water extraction caused by soil shrinkage artefacts.

Experiment II – Root hydraulic conductance

Experimental design

Seeds of spring oilseed rape, (*Brassica napus* L. cv. SW Landmark) were sown into a polypropylene tube (100 mm length x 32 mm inner diameter) loosely packed with a mixture (1:1 v/v) of washed sharp sand and vermiculite (1-3 mm). The growth substrate was selected to provide a high saturated hydraulic conductance during measurements of root hydraulic conductance so that flux of water to the root surface would not be a limiting factor. The tube and growth medium were held within a polythene bag. Spring wheat (*Triticum aestivum* L. cv. Tybalt) seeds were sown into 150 mm long tubes at 50 mm depth to encourage plants to produce a relatively long mesocotyl. Mineral nutrients were provided weekly in 10 ml of half strength Hoagland solution (Epstein 1972) and water was supplied every other day to keep the growth medium moist. Plants were grown in a controlled climate chamber at 18 ± 0.7 °C, 65 ± 9.9 %RH and a light intensity of $175 \pm 16 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation over a 16h photoperiod. The experiment was a completely randomised design. To ensure the growing conditions were uniform, the position of individual plants was changed systematically twice a week (i.e. one position forward and two to the right). Root hydraulic conductance was measured 3 weeks after sowing when plants had two unfolded leaves and a third unfolding. In total, six oilseed rape and six wheat root systems were measured ($n = 6$).

Measurements

Hydraulic conductance was determined by pressurising a detached root system and measuring the efflux of water from the severed hypocotyl (OSR) or mesocotyl (wheat). Between 7 and 9 hours from the start of the light period, the hypocotyl or mesocotyl of an individual plant were cut just below the first leaves, 30 ml of water was added to the polythene bag and the bag plus growth tube and root system were placed in a pressure chamber (ELE international Ltd., Leighton Buzzard, U.K.). The chamber was sealed with the hypocotyl or mesocotyl protruding through a silicone septum in the lid. The chamber was pressurised to 0.3 MPa and the root system left for at least 12 min for a constant water flow to establish from the cut surface. The water collected over this initial period was discarded and efflux over a further 10 min period was measured by absorbing the water expressed from the xylem with a piece of dental cotton roll embedded in a two ml safe-lock Eppendorf tube (see Gallardo *et al.* 1996 for a comparable method). The tube and cotton roll were weighed to the nearest 0.1 mg at the start and end of the efflux period. Hydraulic conductance was calculated as the rate of efflux divided by the chamber pressure. A pilot experiment had established that the flow was constant within 12 min. Some studies have

reported an offset from zero in the flux/water potential or pressure gradient relationship indicating a threshold gradient must be exceeded to initiate water flux (Rieger & Litvin 1999). In our pilot measurements the intercept at zero flux ranged from -0.03 to 0.03 MPa. Forcing the flux/pressure gradient relationship through zero altered the slope by less than 20% compared to a 2 to 3 fold variation in slope between replicate plants. For this reason the main experiment was conducted using a single pressure assuming the slope of the relationship passed through zero. This enabled more replicates to be measured within the time available.

After measurement, the root system was washed from the sand/vermiculite, spread in a transparent dish containing a film of water, scanned with a Régent scanner (LA1600, Quebec, Canada) and analysed with Winrhizo software (Régent, Quebec, Canada). After scanning, the root system was dried in a fan-assisted oven at 70°C until constant mass and the dry weight determined. Leaf area was measured with a leaf area meter (LI-COR, Lincoln Nebraska, USA).

Statistical analyses – Experiments I and II

All statistical analyses were conducted using the software GenStat (v 11.1 2008, VSN International Ltd, Hemel Hempstead, UK). In experiment I the effect of treatments were analysed with a two-way ANOVA test. A repeated measures ANOVA was conducted when the effect of treatments on a variable were assessed on the same experimental unit repeatedly over time or soil depth.

Following ANOVA, relevant means for treatments of interest were compared using the standard error of the difference (SED) between means, on the residual degrees of freedom (df) from the ANOVA, thus invoking the least significant difference (LSD) at the $p = 0.05$ level of significance.

Owing to non-Normality of distribution of residuals, the data from experiment II were analysed using the non-parametric Wilcoxon rank sum test in the R Stats package (v 3.1.1, R Core Team 2014, Foundation for Statistical Computing, Vienna, Austria).

Results

Water extraction and onset of drought

The mean daily VPD ranged between 0.03 and 0.87 kPa during the course of the experiment.

>>INSERT FIGURE 1

Values of daily transpiration rate are an average for the preceding seven day measurement

interval and are plotted at the end point of the interval (Fig. 1). Between 24 and 45 DAS (measurement intervals 17-24 and 38-45 DAS respectively) the transpiration rate of irrigated OSR and wheat increased steadily, from 1.0 ± 0.3 on 24 DAS to 6.4 ± 0.3 on 45 DAS for OSR and 1.1 ± 0.4 to 5.7 ± 0.5 mm/day for wheat. Thereafter, it declined slightly before increasing again over the remainder of the experiment (Fig. 1). From 52 DAS onwards (i.e. interval 45-52 DAS), the rate was significantly greater for irrigated OSR compared to wheat ($P < 0.001$, LSD). On 45 DAS (i.e. interval 38-45 DAS), transpiration rate of non-irrigated OSR and wheat fell below that of the potential rate, represented by their respective irrigated controls ($P < 0.001$, LSD), indicating that the onset of drought occurred at broadly the same time in each species.

Although there was no difference in the timing of onset of drought stress between species (Fig. 1), there was a marginally lower transpiration rate in non-irrigated OSR compared to wheat and significantly more plant available water remaining in the soil for OSR at the onset of the drought response (Fig. 2) ($p < 0.05$, LSD, after repeated measures ANOVA). Between DAS 38 and 45 when the rate of transpiration fell below the potential rate, the plant available water of non-irrigated lysimeters decreased from 38 to 9% v/v for lysimeters planted with wheat and 54 to 23% in lysimeters planted with OSR. This change in soil water content corresponded to a reduction in soil matric potential from -0.31 to -1.31 MPa for wheat and -0.14 to -0.61 MPa for OSR. As there was little available water remaining in the soil profile of non-irrigated wheat lysimeters on day 45 (Fig. 2) there was a significant reduction in the rate of transpiration between 45 and 52 DAS (Fig. 1). The reduction was less pronounced in non-irrigated OSR, presumably because there was a greater amount of soil water that could still be extracted.

Around 52 DAS all plant available water had been extracted from 0 to 80 cm of soil in non-irrigated lysimeters of both species (Fig. 2), but the capacitance probe measurements registered a further decline in soil moisture content from these depths, which could not be completely accounted for by evaporation from the soil surface (as given by the rate of water loss from un-planted columns). We consider the decline in soil moisture content beyond the accepted permanent wilting point (PWP) to be an artefact possibly caused by shrinkage of soil away from the access tube. The matric potential at the final measurement of non-irrigated lysimeters was estimated to be -15.3 MPa, an order of magnitude greater than the PWP. This conclusion is supported by observations of air gaps between the tube and soil in non-irrigated lysimeters, but not irrigated ones when lysimeters were opened up at the end of the experiment. Extraction of water commenced first from the upper layers of soil, followed sequentially from increasingly deeper layers as time progressed (Fig. 3). Oilseed rape began extraction from any given layer on average nearly 5 days later than wheat, and this response was not affected by irrigation regime or soil depth. Thus, there were significant effects of soil depth ($p < 0.001$, $F_{(7,112)} = 229$) and species ($p < 0.01$, $F_{(1,12)} = 12.5$) after a two-way ANOVA with depth as a repeated measure, but no significant effect of

irrigation ($p = 0.17$, $F_{(1,12)} = 2.2$) and no significant interaction between irrigation, species and soil depth ($p = 0.56$, $F_{(7,12)} = 0.75$). Although the onset of water extraction from a particular soil depth occurred later in OSR crops than wheat, the maximum rate of extraction that followed was greater (data not shown).

>>INSERT FIGURE 2 and FIGURE 3

Stomatal conductance

The stomatal conductance of irrigated OSR was greater than wheat on day 74, the difference was nearly three-fold (Fig. 4B). Withholding water resulted in a significant decrease in stomatal conductance in both species. Oilseed rape plants responded to drought by closing their stomata to a greater extent than wheat (85% reduction for OSR of 75% for wheat relative to irrigated controls, $p < 0.001$ for species x irrigation interaction, after 2-way ANOVA, $F_{(1,12)} = 45.37$). The total water use by irrigated OSR was 41% greater than that of wheat (Fig. 1).

The greater water use by OSR was associated with a larger biomass production such that the water use efficiency (WUE) of irrigated OSR and wheat were comparable. Withholding water increased the WUE of OSR to a smaller extent than wheat ($p < 0.05$, $F_{(1,12)} = 5.48$ for species x irrigation, after two-way ANOVA; Fig. 4A). The smaller scale of increase for OSR under drought was observed whether or not apparent water extraction beyond the PWP was included in the calculations.

>>INSERT FIGURE 4

Effects of withholding water on shoot growth

In general the effects of withholding irrigation on the height of wheat plants were small and not statistically significant (Fig. 5). Maximum plant height was reached by 59 DAS and thereafter differences between irrigated and non-irrigated wheat plants diminished. By contrast, OSR plants increased in height at a steady rate over the first 59 days of the experiment, before rapid stem extension increased the height above that of wheat. There was a significant time x species x irrigation interaction on plant height ($F_{(8,128)} = 6.39$, $p < 0.01$ after two-way ANOVA with repeated measures). Withholding irrigation caused a reduction in final height of 21% in OSR, but only a 1% reduction in wheat ($p < 0.01$, LSD, after repeated measures ANOVA).

>> INSERT FIGURE 5

Withholding water reduced the above ground biomass of OSR to a greater extent than that of wheat (Table 1): there was a reduction in biomass of 52% for OSR and only 21% for wheat compared to their irrigated controls. By contrast both the total shoot area (summed area of stems, leaves and pods/ears) and the leaf area of OSR and wheat were reduced to a similar extent (> 50%) by drought. Withholding water not only reduced dry weight of OSR more than wheat, it also reduced pod dry weight significantly more than the ear dry weight of wheat ($p < 0.05$, $F_{(1,12)} = 46.9$, for species x irrigation interaction after two-way ANOVA). While pod dry weight of OSR was halved, wheat ear dry weight was not affected. Thus there was evidence of a change in dry matter partitioning to the ear (ear dry weight:total dry weight) during grain filling in wheat after withholding water, but not to pods in OSR (pod dry weight:total dry weight). The relative water content of the youngest fully expanded leaf was reduced by withholding irrigation to a comparable extent in both wheat and OSR ($p < 0.001$, $F_{(1,12)} = 0.4$, for the main effect of irrigation, Table 1).

>> TABLE 1

The root system

Oilseed rape and wheat produced a comparable RLD when averaged over two soil depths (34-40 and 70-80 cm) and irrigation regimes ($p > 0.05$, $F_{(1-12)} = 0.1$ for species after three way ANOVA), but there was a significant species x depth interaction ($p = 0.01$, $F_{(1-12)} = 6.1$) with wheat locating relatively more of its root length in the deeper soil layer than OSR (Table 2). Withholding water reduced RLD by 24% ($p = 0.01$, $F_{(1-12)} = 0.1$ for irrigation), but the response did not differ between species or with soil depth ($p > 0.05$ for species x irrigation and depth x irrigation interactions).

In general the root surface area density (RSD) followed similar pattern to RLD with wheat plants tending to have a higher RSD at the deeper depth than OSR plants when averaged across irrigation regimes ($p < 0.05$, $F_{(1,12)} = 5.9$ for species x soil depth interaction). However, there was a tendency for RSD of OSR to be more sensitive to soil drying than wheat with OSR showing a 30% reduction in RSD after withholding water compared to little change (<5% increase) in wheat ($p = 0.08$, $F_{(1,12)} = 3.6$ for species x irrigation interaction) (Table 2). There was no species x irrigation x depth interaction effect on RLD or root surface area (Table 2).

The effects of irrigation regime on the distribution of root length density over different root diameter classes is shown in Fig. 6. The results have been averaged for the different species and soil depths. The reduction in RLD with soil drying was the result of reductions in RLD of fine roots (~40% for diameter classes 0-0.1 and 0.1-0.2 mm ($p < 0.05$, LSD, after three-way ANOVA, split-plot for soil depth effect)). However, this was offset to a small extent

by an accompanying increase (~40%) in RLD of the 0.2-0.3 mm diameter class ($p < 0.1$, LSD).

>>INSERT FIGURE 6 and TABLE 2

Root hydraulic conductivity

In experiment II the whole root system conductance of young OSR plants was two and a half times that of wheat ($p < 0.01$, Wilcoxon rank sum test; Table 3). Plants of both species had a comparable shoot dry weight, but the shoot area and total root length were significantly greater for OSR ($p < 0.01$ after Wilcoxon rank sum test), whilst the average root diameter and root dry weight were smaller. The specific root length (length per unit root dry weight) of OSR was over twice that of wheat. The leaf area:root length ratio of OSR was 43% greater than that of wheat. As a result of these differences in plant morphology, the root hydraulic conductivity of OSR and wheat expressed per unit of root length did not differ significantly ($p = 0.18$, $W = 9$, Wilcoxon rank sum test), but when expressed per unit root surface area, root hydraulic conductivity of OSR was double that of wheat ($p < 0.05$, $W = 4$, Wilcoxon rank sum test, Table 3).

>>INSERT TABLE 3

Discussion

Oilseed rape is more drought sensitive than wheat

The results show that OSR was more sensitive to drought than wheat in this experiment. Although the onset of drought occurred at about the same point in time for both species, the effect of a restricted supply of water on OSR growth and yield parameters was greater. The onset of drought can be defined by reference to a number of physiological processes (Sadras & Milroy 1996). Here we define it as the time at which the transpiration rate falls below the potential rate measured when the water supply is unlimited, and is taken to represent the point at which stomata respond to soil drying (Day *et al.* 1978; Sadras & Milroy 1996). In the current experiment this occurred between 38 and 45 days after sowing (Fig. 3). As transpiration measurements were made on a seven day interval basis, it is not possible to be more precise in our estimates. During this period the transpiration rate of non-irrigated

OSR was limited when there was between 54 and 23% of plant available water left in the soil, while for wheat only between 38 and 9% remained (Fig. 2). These threshold values are broadly in line with those reported previously for wheat and other species from field, lysimeter and pot experiments (Meyer & Green 1980; Sadras & Milroy 1996). The results suggest that the stomata of OSR may be more sensitive to soil drying than those of wheat and begin to close, thereby reducing transpiration below the potential rate, when there is a greater amount of available water remaining in the soil profile. However, direct measurements of stomatal conductance during the progress of soil drying will be necessary to substantiate this hypothesis. Any difference in sensitivity is likely to result from complex causes and could involve species differences in hydraulic and non-hydraulic signalling in conjunction with differences in root distribution and transpiration rate (Denmead & Shaw 1962; Tardieu & Davies 1993; Sadras & Milroy 1996).

Under the current experimental conditions, OSR plants had a greater water requirement than wheat (Fig. 1), when this requirement was not met there was a greater effect of water limitation on growth. The total above ground biomass was reduced to a larger extent in OSR than wheat, although there was no difference between species in response of leaf or shoot area expansion to drought. The growth of reproductive organs seemed to be particularly sensitive in OSR. For instance, whilst wheat ear dry weight was not reduced by withholding water, pod dry weight of OSR was halved (Table 1). There have been few studies comparing the sensitive of reproductive growth in OSR with that of other species. However, *Brassica napus* has been reported to be considerably more sensitive to water deficits occurring around anthesis and seed filling compared to mustard (*B. juncea*), which may be related to its inferior ability to adjust osmotically (Ma *et al.* 2006). Interestingly, stem extension appeared to be relatively insensitive to soil drying compared with other aspects of growth. Plant height continued to increase in non-irrigated OSR and wheat after plant available water appeared to have been depleted, and significant reductions in final plant height were only observed in OSR (Fig. 5). The plant height measurements made here will have included contributions from both stem and leaf extension, although in the second half of the experiment when drought severity was at its greatest, stem extension is likely to have dominated as final leaf emergence will have occurred.

The greater water requirement of OSR relative to wheat in irrigated lysimeters was associated with a larger biomass production. Consequently, the WUE of OSR and wheat did not differ significantly when water availability was unrestricted (Fig. 4A). The values of WUE are comparable with those reported for field-grown crops (Foulkes *et al.* 2001). Withholding water increased the WUE of both species, a common response to drought (Foulkes *et al.* 2001), but the increase in WUE of OSR was smaller. Differences in the sensitivity of the two species to drought was less pronounced in root growth. RLD of OSR was reduced by 37% compared to 10% for wheat with a similar difference in RSD, but for each the species x irrigation interaction was not statistically significant ($p = 0.10$, $F_{(1,12)} = 3.12$ and 0.08 , $F_{(1,12)} =$

3.6, respectively). There were indications of some plasticity in growth of both wheat and OSR roots in response to soil drying. Thus, the length of roots in diameter classes 0.2 - 0.3 mm were increased ($p < 0.001$, LSD) offsetting to some extent the reduction in length of the finer root classes (Fig. 6). This may reflect an increased extension of primary and 1st order lateral roots and a reduction in initiation and/or extension of higher order laterals.

Oilseed rape extracts water as effectively from the soil as wheat

In all lysimeters roots had reached the base by the end of the experiment and thus there was no difference in maximum measured rooting depth between OSR and wheat. Moreover, the total root length density in the deepest layer measured (70-80 cm) was comparable for wheat and OSR under both irrigated and non-irrigated conditions, indicating that when soil conditions are favourable OSR is able to generate a large root system deep in the soil profile - Although there was no evidence that OSR had a shallower root system than wheat at the end of the current experiment, OSR began to extract water from each soil layer several days after wheat (Fig. 3). This may reflect species differences in the rate of root growth and soil exploration, with the roots of OSR reaching deeper layers later than wheat and/or differences in root hydraulic architecture.

The hydraulic conductivity of OSR roots in the current study, measured on a surface area basis, was about twice that of wheat. In an investigation of five species with contrasting roots, root hydraulic conductivity (per unit surface area) was inversely related to root diameter and cortex width, suggesting that species with thinner roots or roots with a narrow cortex had the highest conductivity (Rieger & Litvin 1999). Further observations suggested that the cortex width was a stronger determinant of conductivity than root diameter (Rieger & Litvin 1999). The radial pathway across the root cortex has long been considered to offer the greatest resistance to the flow of water through the plant (Burch 1979; Passioura 1980; Rieger & Litvin 1999). Historically, the major resistance was believed to be located at the endodermis and, if present the exodermis, where apoplastic flow is restricted by suberised Casparian bands and where water is forced to traverse cell membranes or travel symplastically to reach the xylem. However, more recent evidence suggests that in some species water may flow preferentially via a symplastic or cell to cell pathway (Rieger & Litvin 1999; Steudle 2000). There is also growing evidence that the flow of water across cell membranes may be controlled by aquaporins, the numbers and gating of which may be regulated according to the physiological state of the roots by factors such as cytoplasmic pH and abscisic acid (Bramley *et al.* 2007; Tardieu *et al.* 2010). Thus the greater hydraulic conductivity of OSR roots observed in the present study compared to wheat may result from their smaller diameter and hence shorter radial path length and possibly narrower cortex. However, species differences in the number and activity of aquaporins could also be

involved.

When expressed on a root length basis, the hydraulic conductivity of OSR and wheat roots did not differ significantly ($p = 0.18$, $W = 9$, Wilcoxon rank sum test, $n = 6$). Differences were smaller when expressed in this way because OSR roots had a smaller surface area to root length ratio. Per unit root length the conductivity was $0.16 \times 10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ for wheat and $0.22 \times 10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ for OSR, which corresponds favourably to values reported in the literature: $0.11 \times 10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ for wheat and $0.41 \times 10^{-10} \text{ m}^3 \text{ s}^{-1} \text{ m}^{-1} \text{ MPa}^{-1}$ for lupin (Gallardo *et al.* 1996).

Although non-irrigated oilseed plants started to take up water from each soil layer about a week later than wheat plants (Fig. 3), the maximum uptake rate by OSR was, for most soil layers, greater than that of wheat (data not shown). Thus OSR was eventually able to extract all the potentially available water from each soil depth. Collectively these results suggest that OSR was as effective as wheat in extracting water from the soil and that its greater sensitivity to drought was not the result of inferior root growth and soil exploration.

Differences in water use of the species appeared to be related more closely to differences in the rate of water influx per unit root length and stomatal conductance per unit leaf area rather than the total root length and canopy area per se. In irrigated lysimeters the influx of water per unit root length of OSR measured towards the end of the experiment (data not shown) was nearly twice that of wheat ($6.4 \text{ cf } 3.3 \times 10^{-15} \text{ m}^3 \text{ H}_2\text{O m}^{-1} \text{ root length s}^{-1}$ respectively) averaged across both the soil depths measured. If it is assumed that the hydraulic conductivity per unit root length was the same as that measured in young plants in experiment II, then the potential difference between soil and xylem required to drive the observed influx is 1.3 times greater for OSR than for wheat (average across soil depths). The greater potential difference estimated for OSR is consistent with its greater stomatal conductance and lower leaf relative water content at this time (Fig. 4 and Table 1).

Implications for oilseed rape crops

Oilseed rape and wheat were sown in the lysimeters at plant population densities and with a fertilizer regime equivalent to those used for field crops in the UK. In spite of this, plant growth per unit area of ground was greater than would normally be found in the field. Several factors are likely to have contributed to the extensive growth. Lysimeters were packed with a top soil relatively high in organic matter, which may have supplied large quantities of N through mineralisation at all soil depths, in addition to the N applied in fertilizer. Packed lysimeters often permit more extensive root growth than those utilising undisturbed soil cores (Meyer *et al.* 1990). In addition, the shade meshing placed around the canopy may not have fully compensated for the lack of competition from neighbouring plants. The exposed nature of the isolated and large canopies is likely to have contributed to the high rates of

transpiration observed, as vapour pressure deficits in the open-sided glasshouse were not dissimilar to those recorded in field experiments in the UK (Werken & Jaggard 1998). The relatively high rates of transpiration as seen in irrigated OSR from DAS 65 onwards, are unlikely to be found in the field under UK conditions, because in closed crop canopies the influence of stomata on canopy transpiration is less and the contribution of boundary layer resistance greater than in isolated plants or small populations (Jarvis & McNaughton 1986). However, in spite of the large canopies and rates of transpiration in the lysimeters, water use efficiencies of irrigated and non-irrigated wheat plants (Fig. 4) were similar to those found in the field, namely, 3.72-4.43 and 4.89-6.31 g DW m⁻² mm⁻¹, respectively, in a UK field study by Foulkes *et al.* (2001), suggesting that the observed responses to drought are representative of field crops. Total water use of OSR and wheat in this experiment (Fig 1) was also comparable to total crop water use in field studies, although the irrigated OSR water use was relatively high. In a three year UK field experiment with wheat, the irrigated wheat water use was 383-438 mm (Foulkes *et al.* 2001). For OSR, as far as we know no UK values are readily available, generally total water use reported in the literature is lower than the 498 mm reported here, but in a Tasmanian field study, irrigated OSR water use was similar, namely 488 mm (Rao & Mendham 1991).

The bench marking of OSR against wheat in this study has provided important new information about the hydraulic properties of OSR and its relative sensitivity to drought. The similarity in root hydraulic conductivity when expressed on a unit length basis between wheat and OSR suggests that the critical RLD for easy extraction of plant available water will be comparable. The concept of a critical RLD is used in modelling water capture by root systems and for quantifying the potential benefits of improving root systems (King *et al.* 2003; Bingham 2005). As their root distributions and critical RLDs may be quite similar (Barracough 1989; Barracough & Leigh 1984; Liu *et al.* 2011a;b), a given increase in RLD in the subsoil would provide both species with access to the same amount of additional stored water (King *et al.* 2003; Bingham 2005). However, depending on the relative contribution of stomata to the control of canopy transpiration in the field, our results suggest that increasing access to soil water reserves could have a greater impact on the yield of OSR compared to wheat in low rainfall areas, as stomatal conductance and growth of reproductive organs appear to be more sensitive to drought in OSR. A reduction in sensitivity of stomata and growth of pods and seeds to restricted water supply, possibly through an improvement in the capacity for osmoregulation, could be a complimentary strategy for improving OSR yield under drought as it would allow plants to photosynthesise at high rates for longer as the soil dries (Ma *et al.* 2006).

Conclusions

By benchmarking OSR against wheat, this study has shown that OSR is more sensitive to soil drying than wheat. The crop's response to drought happened at the same moment in time for OSR and wheat, but at a lower soil water deficit for OSR. The greater drought sensitivity of OSR does not result from an inferior root growth and activity. Root growth, distribution and root hydraulic conductivity per unit root length were comparable in OSR and wheat. The results imply that the critical root length density for water extraction by OSR (minimum RLD required for extraction of all the plant available water) will be similar to wheat. The greater sensitivity to drought, compared to wheat, was associated with a lower WUE and a greater reduction in stomatal conductance and growth of reproductive organs in OSR following soil drying.

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Captions to figures and tables

Figure 1

Transpiration rates per lysimeter for oilseed rape (OSR) and wheat, irrigated and non-irrigated. Data points are the average daily rate for the measure interval (usually seven days), plotted as means of five replicate lysimeters at the end of the measurement interval. The vapour pressure deficit of the atmosphere (open circles) measured at plant height is plotted on the secondary y-axis. There was a significant interaction of species x irrigation x time ($F(11,176) = 26.8$, $p < 0.001$ after two-way ANOVA with repeated measures). Vertical bar shows $LSD_{0.05}$ for species x irrigation x time interaction ($df = 97.2$).

Figure 2

Change in plant available water in the soil (0-80 cm depth) expressed as a % of the total over time for non-irrigated oilseed rape and wheat. Total plant available water is the amount of water held by the soil between water content on DAS 0 and permanent wilting point (water content at -1.5 MPa). Broken line indicates apparent water extraction from soil at matric potentials beyond -1.5 MPa (likely an artefact created by shrinkage of soil). The vertical bar shows $LSD_{0.05}$ ($df = 20.5$) for species x time interaction effect after one-way ANOVA with repeated measures for DAS 0-52 ($F(8.64) = 6.1$, $p < 0.01$).

Figure 3

Start day of water extraction from different soil depths for lysimeters planted with oilseed rape (OSR) or wheat, averaged across irrigation treatments. Bars are means of five replicate lysimeters. The inset shows how start date of uptake from a soil layer was estimated by interpolation between measured values of volumetric soil moisture content. Only the effect of species is significant ($F(1,12) = 12.49$, $p < 0.01$, after two-way ANOVA with repeated measures for depth). None of the interactions are significant, nor is the effect of irrigation. $LSD_{0.05}$ shown is for the depth x species interaction ($F(7,112) = 1.95$, $p = 0.12$).

Figure 4

4A: Water use efficiency (WUE), mean values shown ($n=5$), calculated from above ground biomass and cumulative water loss by evapotranspiration over the whole experiment. Adjusted WUE values are also presented; for the adjusted values it was assumed that no water was taken up by plants below a soil matric potential of -1.5 MPa. The interaction of species and irrigation was significant: $F(1,12) = 5.48$, $p = 0.04$. The plotted $LSD_{0.05}$ bar represents the species x irrigation interaction ($df = 12$); this is the $LSD_{0.05}$ after two-way ANOVA, for non-adjusted data.

4B: Leaf stomatal conductance (g_s) measured 74 days after sowing. Values are means of

five replicates. After a two-way ANOVA, the interaction of species and irrigation was significant: $F(1,12) = 45.37$, $p < 0.001$. Vertical bar is the $LSD_{0.05}$ for irrigation x species interaction ($df = 12$).

Figure 5

Average plant height of oilseed rape and wheat as affected by irrigation treatment. Points are means of five replicates lysimeters with three plants measured per lysimeter. There was a significant interaction of time x species x irrigation; $F(8,128) = 6.39$, $p < 0.01$ after two-way ANOVA with repeated measures. Vertical bar shows $LSD_{0.05}$ for time x species x irrigation interaction, $df = 49.7$.

Figure 6

Root length density (RLD) distribution (in cm cm^{-3} soil), over four root diameter classes (root diameter in mm). Mean of species (OSR and wheat) and soil depths (40 and 80 cm) for irrigated and non-irrigated plants are shown ($n = 20$). Measurements were made 84 and 85 DAS for wheat and OSR respectively. A three-way ANOVA was carried out, split-plot for soil depth effect. There was a significant interaction of diameter class x irrigation ($F(3,96) = 29.84$, $p < 0.001$, $LSD_{0.05} = 0.198$). Vertical bar shows $LSD_{0.05}$ for diameter class x species interaction. There was also a significant interaction of depth x diameter class x species ($F(3,96) = 3.23$, $p < 0.05$).

Table 1 Growth parameters and relative water content of total number of plants harvested per lysimeter. Plants were harvested on days 84 and 85 after sowing for wheat and oilseed rape (OSR) respectively. Values are means and s.e.m. of five replicate lysimeters. P-values are for main effects and interactions after two-way ANOVA; $LSD_{0.05}$ for $df = 12$ given when main effect or interaction was significant.

Table 2 Root length density and surface area at two soil depth intervals and influx rate of water estimated between DAS 73 and 80 (irrigated plants only). Values are means and s.e.m. of five replicate lysimeters. P values are for Species (S), Irrigation (I) and Depth (D) effects and their Interactions, after three-way ANOVA for root data and two-way ANOVA for influx data. $LSD_{0.05}$ given when main effect or interaction was significant.

Table 3 Root and shoot parameters of oilseed rape and wheat plants measured for root hydraulic conductivity in Experiment II. Values are means and s.e.m. of six replicate plants for each species. The test statistic (W) and the p-value of a Wilcoxon rank sum test for difference between oilseed rape and wheat medians are given in the last two columns.

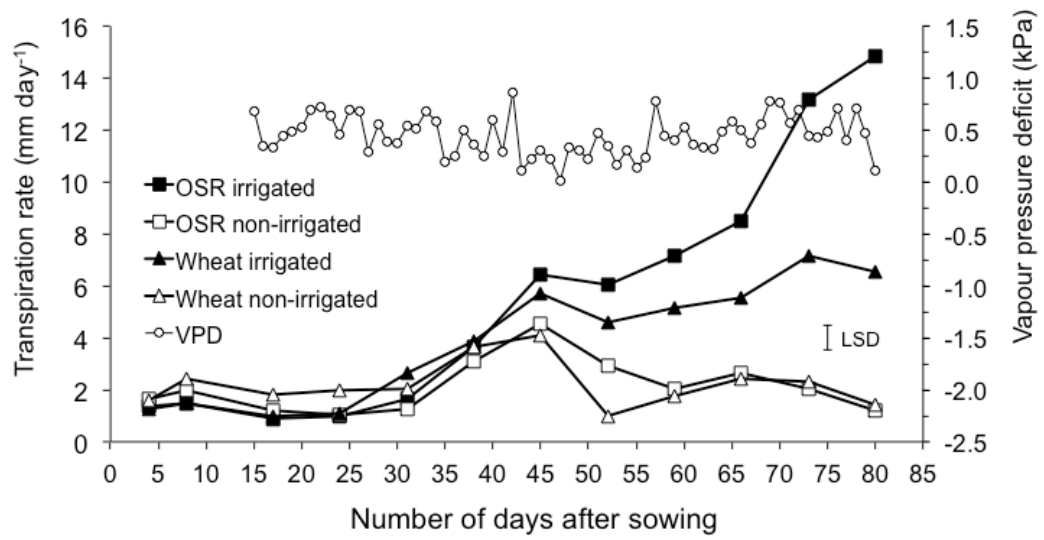


Fig. 1

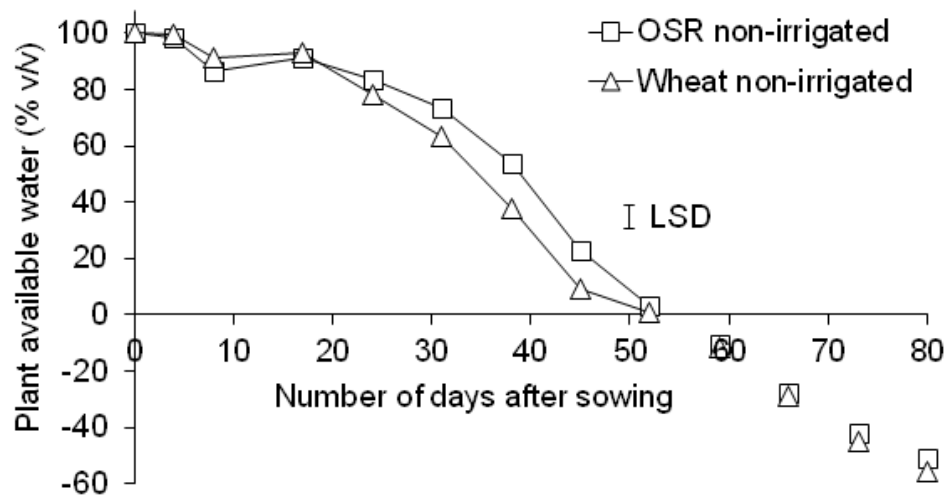


Fig. 2

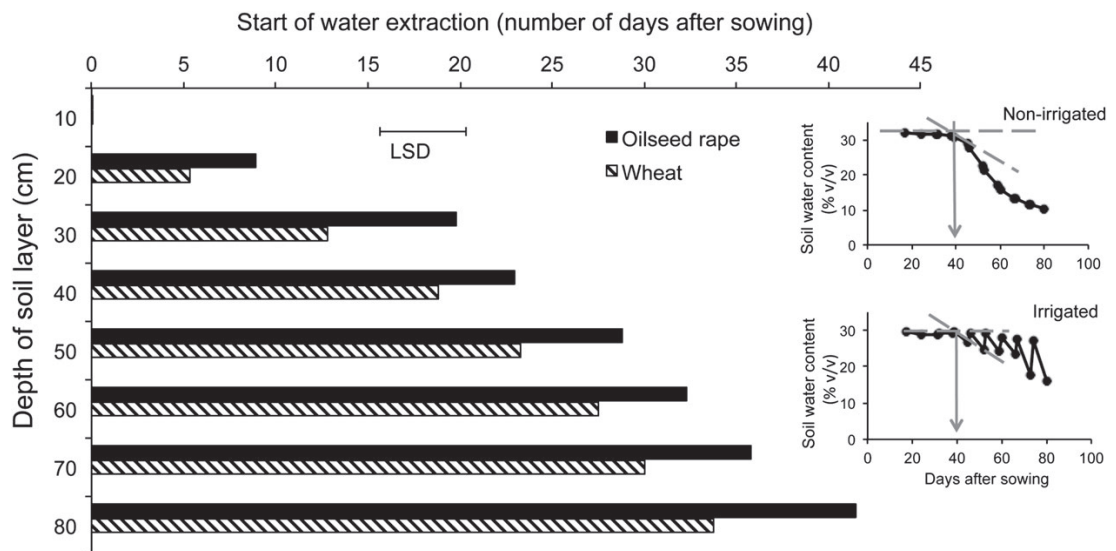


Fig. 3

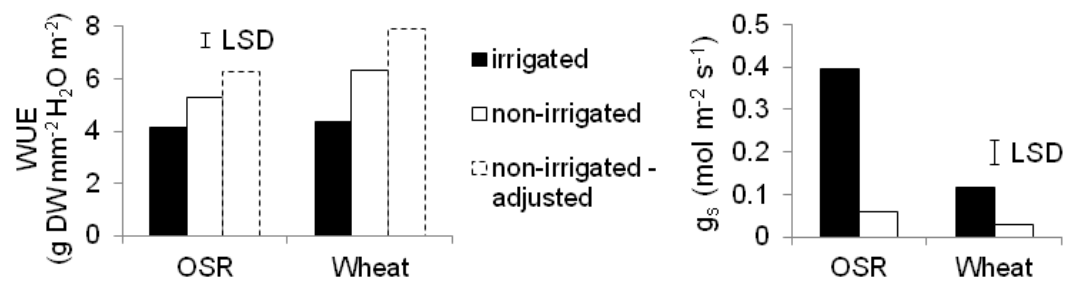


Fig. 4

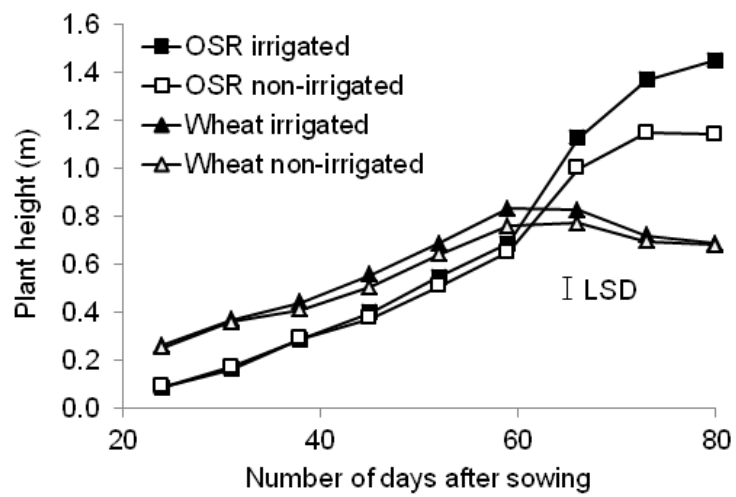


Fig. 5

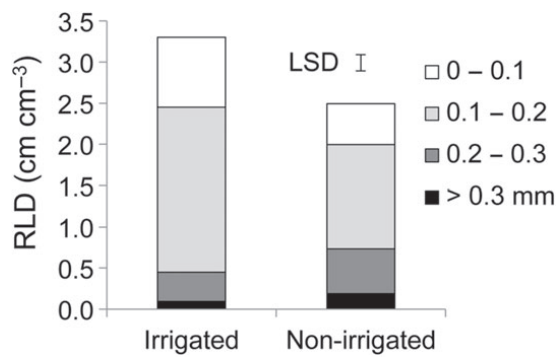


Fig. 6

Table 1 Growth parameters and relative water content of total number of plants harvested per lysimeter. Plants were harvested on days 84 and 85 after sowing for wheat and oilseed rape (OSR) respectively. Values are means and s.e.m. of five replicate lysimeters. P-values are for main effects and interactions after two-way ANOVA; LSD_{0.05} for df =12 given when main effect or interaction was significant.

	OSR	OSR	Wheat	Wheat	ANOVA test statistics			LSD _{0.05}	LSD _{0.05}	LSD _{0.05}
	Irrigated	Non-irrigated	Irrigated	Non-irrigated	Species (S)	Irrigation (I)	S x I	S	I	S x I
Total above ground biomass (g)	144.9 ± 4.8	69.8 ± 1.9	108.5 ± 4.1	86.1 ± 3.1	p = 0.03 F _(1,12) = 6.30	p < 0.001 F _(1,12) = 149.5	p < 0.001 F _(1,12) = 43.7			12.7
Total shoot area (cm ²)	8170 ± 639	3032 ± 136	7920 ± 511	3824 ± 172	p = 0.47 F _(1,12) = 0.55	p < 0.001 F _(1,12) = 158.2	p = 0.18 F _(1,12) = 2.02		800	
Total leaf area (cm ²)	5363 ± 596	1756 ± 89	5970 ± 428	2525 ± 325	p = 0.08 F _(1,12) = 3.56	p < 0.001 F _(1,12) = 93.6	p = 0.83 F _(1,12) = 0.05		794	
Total pod or ear DW (g)	45.6 ± 3.0	21.9 ± 0.8	26.0 ± 0.8	25.2 ± 1.0	p < 0.001 F _(1,12) = 53.8	p < 0.001 F _(1,12) = 23.5	p < 0.001 F _(1,12) = 46.9			5.2
Relative water content (%)	0.84 ± 0.04	0.68 ± 0.06	0.93 ± 0.01	0.63 ± 0.04	p = 0.56 F _(1,12) = 30.9	p < 0.001 F _(1,12) = 0.4	p = 0.12 F _(1,12) = 2.8		0.09	

Table 2 Root length density and surface area at two soil depth intervals and influx rate of water estimated between DAS 73 and 80 (irrigated plants only). Values are means and s.e.m. of five replicate lysimeters. P values are for Species (S), Irrigation (I) and Depth (D) effects and their Interactions, after three-way ANOVA for root data and two-way ANOVA for influx data. LSD_{0.05} given when main effect or interaction was significant.

	Depth (cm)	OSR	OSR	Wheat	Wheat	ANOVA test statistics						
		Irrigated	Non- irrigated	Irrigated	Non- irrigated	Species (S)	Depth (D)	Irrigation (I)	S x D	S x I	D x I	S x D x I
Root length density (cm cm ⁻³)	30-40	3.85 ± 0.49	2.11 ± 0.22	2.67 ± 0.20	2.38 ± 0.15	F _(1,12) = 0.1	F _(1,16) = 1.9	F _(1,12) = 8.2	F _(1,16) = 6.1	F _(1,12) = 3.1	F _(1,16) = 1.0	F _(1,16) = 1.1
	70-80	3.18 ± 0.25	2.31 ± 0.25	3.51 ± 0.60	3.18 ± 0.22	p = 0.79	p = 0.19	p = 0.01	p = 0.03	p = 0.10	p = 0.34	p = 0.30
	LSD _{0.05} (df)							0.62 (12)	0.73 (23.8)			
Root surface area density (cm ² cm ⁻³)	30-40	0.22 ± 0.03	0.13 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	F _(1,12) = 1.9	F _(1,16) = 4.1	F _(1,12) = 1.9	F _(1,16) = 5.9	F _(1,12) = 3.6	F _(1,16) = 2.9	F _(1,16) = 0.6
	70-80	0.19 ± 0.01	0.16 ± 0.02	0.22 ± 0.04	0.24 ± 0.01	p = 0.20	p = 0.06	p = 0.20	p = 0.03	p = 0.08	p = 0.11	p = 0.46
	LSD _{0.05} (df)								0.05 (22.9)			

Table 3 Root and shoot parameters of oilseed rape and wheat plants measured for root hydraulic conductivity in Experiment II. Values are means and s.e.m. of six replicate plants for each species. DW, dry weight. The test statistic (W) and the p-value of a Wilcoxon rank sum test for difference between oilseed rape and wheat medians are given in the last two columns.

	Oilseed rape (n=6)	Wheat (n=6)	W	P-value
Root DW (g)	0.023 ± 0.002	0.036 ± 0.003	1	<0.01
Root length (cm)	1345.0 ± 93.9	792.9 ± 67.2	1	<0.01
Root surface area (cm ²)	95.7 ± 7.3	85.2 ± 8.0	12	0.39
Root diameter (mm)	0.224 ± 0.006	0.328 ± 0.007	0	<0.01
Shoot DW (g)	0.090 ± 0.008	0.066 ± 0.007	7	0.09
Shoot area (cm ²)	19.6 ± 1.2	8.0 ± 0.2	0	<0.01
Specific root length (m g ⁻¹)	589 ± 25.0	228 ± 24.9	0	<0.01
Leaf area/root length (cm ² m ⁻¹)	1.50 ± 0.132	1.05 ± 0.094	4	0.03
Total root system conductance (m ³ s ⁻¹ MPa ⁻¹)	2.8 ± 0.32 × 10 ⁻¹⁰	1.2 ± 0.22 × 10 ⁻¹⁰	0	<0.01
Conductivity (m ³ s ⁻¹ MPa ⁻¹ m ⁻¹ root length)	2.2 ± 0.39 × 10 ⁻¹¹	1.6 ± 0.31 × 10 ⁻¹¹	9	0.18
Conductivity (m ³ s ⁻¹ MPa ⁻¹ m ⁻² root surface area)	3.1 ± 0.55 × 10 ⁻⁸	1.5 ± 0.29 × 10 ⁻⁸	4	0.03